The Relevance of Rabies to Today's Military

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A 24-year-old Army Specialist was assigned as a cook at Combat Base Chamkani, Paktia Province, Afghanistan, from May 2010 to May 2011.[1] He was a known animal enthusiast and had been caring for unauthorized dogs in his unit's area, in spite of General Order Number 1 which forbade the keeping of animals in theater. Feral dogs have been a perennial issue throughout the combat theaters of Iraq and Afghanistan, as they are attracted to the presence of food waste at dump sites around the bases.[2] During an attempt to break up a dog fight between one of the local unauthorized dogs and a feral dog, the Specialist was bitten on the hand. According to reports, the Specialist told his mother that he was ordered to shoot the feral dog and have it sent for rabies testing. It has also been reported that a rabies vaccine series was initiated, but discontinued because the vaccine had expired. Unfortunately, there is no evidence that any animal was tested for rabies and no record of medical treatment being sought or given. The Specialist left Afghanistan in May 2011 with his unit for Germany. He later reported to his new assignment at Fort Drum, New York, in August 2011. By this time, he had started to exhibit neurologic symptoms. He complained of a tingling pain radiating down his arm on August 14, and was treated for tendonitis at a civilian hospital. He reportedly had trouble drinking on August 17 and collapsed at work on August 19, again seeking treatment at a civilian hospital. At this point, it was learned that he had received the dog bite in Afghanistan. This history and the symptoms led to the suspicion of rabies, which triggered specific testing at Wadsworth Center, the New York State Department of Health's public health laboratory. Rabies virus antigens were detected in hair follicles of nuchal skin biopsy specimens by direct immunofluorescence, and rabies viral RNA was found in saliva and cerebrospinal fluid (CSF) by reverse transcriptase–polymerase chain reaction. Once rabies was identified as a potential cause of his symptoms, a coma was medically induced as part of an experimental rabies treatment protocol. At the time of his hospitalization, rabies virus specific immunoglobulin M and immunoglobulin G antibodies were detected in his serum and cerebral spinal fluid in laboratory testing performed by the Wadsworth Center and at the Centers for Disease Control and Prevention (CDC).[3] Virus neutralizing antibodies were found in the serum at a level of 0.07 IU/mL on August 28, and by the date of his death on August 31, had increased to 0.50 IU/mL. Virus neutralizing antibody was not detected in the CSF. Laboratory testing at the CDC detected no rabies virus neutralizing antibody in the blood drawn in May 2011 as a routine banked sample upon his return from deployment. He suffered a massive brain hemorrhage and succumbed to rabies on August 31. He was the first US military member to die from rabies since the Vietnam war.[1] Postmorten tests performed at the CDC established that the virus variant was consistent with the canine rabies virus found in Afghanistan.[3]

The Army and local health departments, in collaboration with the New York State Department of Health and the CDC, interviewed and provided risk assessments to the patient's family members and friends, fellow travelers, health care workers, and any other personnel who may have interacted with the patient.[3] Any individual that was identified as meeting the exposure criteria set by the Advisory Committee on Immunization Practices was given postexposure prophylaxis. The exposure criteria includes wound or mucous membrane exposure to the patient's saliva, CSF, neural tissue, or tears.[4] The Army Public Health Command assembled a rabies response team to locate other members of the Soldier's unit who may not have reported bites to medical providers while in theater. More individuals were identified through medical record reviews. Any Soldier who had a possible animal exposure was evaluated and, where indicated, given postexposure prophylaxis, which typically consists of immediate administration of rabies immune globulin, followed by a series of 4 antirabies vaccinations given on days 0, 3, 7, and 14.[5] Postexposure prophylaxis has proven to be very effective when given promptly, however, rabies is considered to be universally fatal once clinical signs develop. In all, about 9,000 personnel were evaluated to assess their risk of rabies exposure.[1]

REPORTED ANIMAL BITES

The Armed Forces Health Surveillance Center (AFHSC) reported 643 animal bites to US personnel in Southwest Asia and the Middle East combat operational theaters from 2001 through 2010, half of those being dog bites.[6] A total of 117 personnel reported that they received some amount of rabies vaccine. Of the 20,522 total animal bites reported from US Armed Forces worldwide during that period, the majority of the reports from outside

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the current combat operational theaters were from dog bites.[6]

Rabies has been known since ancient times, with the first basic treatment for rabies demonstrated by Louis Pasteur in 1885. Still, as of 2001, every 15 minutes one person died from rabies and over 300 were exposed.[7] Most exposures to rabies worldwide are due to bites from canines. Worldwide, the rabies virus kills an estimated 55,000 people annually, with about 56% of the deaths in Asia and 44% in Africa.[8] The majority (84%) of these deaths occur in rural areas. In many of these places, there are often great difficulties to overcome in the transport of medical supplies, especially those requiring cold storage. Worldwide, there is a limited supply of rabies immune globulin.[9]

SURVIVORS ARE EXCEPTIONS

Rabies is considered to be the infectious disease with the highest case-fatality ratio.[10] There have been only a handful of recent cases in which a patient has survived after symptoms were exhibited, each having had circumstances that may have weighed in the patient's favor. As described by Willoughby et al,[11] in 2004, a girl aged 15 received a bite on the finger while catching and releasing a bat from inside a building in Wisconsin. The finger was washed and disinfected with hydrogen peroxide. No medical attention was sought at the time and no post exposure prophylaxis was administered. A month later, she felt symptoms including nausea and vomiting and sought medical attention. Her symptoms progressed to include fever, salivation, and difficulty in swallowing. Laboratory tests of serum and cerebral spinal fluid showed the presence of antirabies antibodies. As part of a new and aggressive treatment regime, the patient was placed in an induced coma using ketamine, phenobarbital, and midazolam. Ribavirin and (later) amantadine were added for direct antiviral effect. After about 2 weeks of aggressive therapy, the girl began to show positive effects. She was considered to be clear of transmissible rabies by day 31 of hospitalization and was discharged on day 76. The girl initially had severe neurological impairment which has progressively, albeit slowly, improved with time. It is possible that the therapy in this case was more successful than in other cases due to a possible lower dose of rabies virus, early recognition of the symptoms, and aggressive management. The therapy was based on a hypothesis of protecting the brain from injury while enabling the immune system to mount a response and clear the virus. This treatment regime has since been termed the "Milwaukee Protocol." According to the Children's Hospital of Wisconsin Rabies Registry,[12] 4 of 28 rabies patients treated using the Protocol have survived (as of this writing). There is debate among scientists concerning how many of surviving patients actually responded to the therapy, as well as to the appropriate advice for treatment of future patients.[13]

The rabies virus spreads throughout the body through the cells of the nervous system. It is usually introduced into the tissue through a bite, scratch, or mucous membranes from an inoculum of saliva. The virus may remain in the local tissue for some period of time depending on the depth of the wound, innervation to the area, and other biological factors. Upon reaching nerve cells, the virus spreads very quickly and efficiently through the central nervous system, by fast axonal transport along neuroanatomical pathways.[13] The virus travels in a retrograde direction through the axon toward the neuron cell body. The rabies virus is able to take advantage of the axonal transport machinery of the neuron to travel through the axon at speeds of 3 to 10 mm per hour.[14] Once the virus has passed through the brainstem into the brain, the antirabies immunoglobulin is unable to reach the virus, because it cannot pass through the blood brain barrier. Neurologic symptoms are exhibited once the virus passes into the brain. After reaching the brain, the virus then travels outward to the salivary glands where it is available for transmission to the next victim. The rabies virus has evolved a unique and extremely effective mode of transmission, combining placement of the virus with alteration of mammalian behavior to allow it to be passed on to other victims.

US ARMY EXPERIENCE WITH RABIES

The Army has long recognized the threat of rabies to military operations. The Office of The Surgeon General received reports in 1937 of:

> …5,962 dogs, 225 cats, 18 monkeys, 1 fox, and 1 squirrel that were given single dose antirabic vaccination at 67 Army posts during the calendar year 1937. Quarterly Veterinary Sanitary Reports showed that vaccination is practiced at 71 percent of the reporting stations.[15]

According to the 1937 report, the last reported human case of rabies in the military was in 1926. There were 8 cases and 6 deaths reported from 1906 to 1926.[15]

In 1942, the Eighth Service Command Medical Laboratory was established at Fort Sam Houston, Texas.[16] It included a food analysis branch and a virus laboratory, both of which were supervised and operated by veterinary personnel. The virus laboratory was mainly concerned with the study of troop health aspects of such diseases as typhus, Rocky Mountain spotted fever, lymphogranuloma venereum, lymphocytic choriomeningitis, rabies, ornithosis, equine encephalomyelitis, and St. Louis encephalitis. The various Army veterinary

laboratories across the continental United States eventually combined into the Department of Defense Food Analysis and Diagnostic Laboratory (FADL). In 2011, the Army Veterinary Command was merged into the newly established US Army Public Health Command (USAPHC), and the FADL became part of the USAPHC Region-South. Currently, there are 2 Army laboratories with rabies testing capabilities, one at the USAPHC Region-South in Fort Sam Houston, and the other at the USAPHC Region-Europe in Landstuhl, Germany.

ANIMAL HOSTS

The primary carrier for rabies (genotype 1 of the genus *Lyssavirus*) worldwide is canine. This is especially true in urban centers of the developing world. It should be understood, however, that any mammal can become infected by the rabies virus. There are currently 9 recognized antigenic variants of the rabies virus. Each variant is associated with a specific species of mammal that commonly carries the virus within a certain region. This relationship is complex and the genetic characterization of the variants is being redefined as the science continues to advance. Additionally, the habitats of the host species are constantly changing. For example, raccoon rabies in the states of the US eastern seaboard has been slowly moving westward. With wildlife vaccination efforts in Texas, the canine variant of rabies in coyotes has been eliminated in Texas, but persists south of the border in Mexico. Further, it has been reported in Mexico that the bat variant in vampire bats has been found at higher elevations of the mountains due to an increase in average air temperature. Mongoose throughout the Caribbean islands, where they are an invasive species, are now demonstrating a unique challenge in regards to rabies. They have been a particular problem in Puerto Rico, where they not only pose a threat of rabies, but also threaten endangered birds through predation.[17]

In 1942, Fox wrote of the presence of bat rabies in Trinidad.[18] He emphasized the need for inoculation of men and cattle, but also emphasized that rabies must be kept in perspective compared with other communicable diseases found in the tropics. Rabies had been recognized earlier in the century in cattle in Brazil and was linked with vampire bats. Rabies is an increasing economic issue throughout Central and South America due to the impact of virus transmission from vampire bats to cattle and other livestock. It should be noted that bat control efforts may inadvertently have an adverse impact by the decimation of collocated beneficial bat species.[19] Many species of bats cohabit the same roosts as vampire bats. Insectivorous bats are beneficial through the feeding on vast numbers of insects, while many plants depend on the pollinating activities of fruit bats.[20]

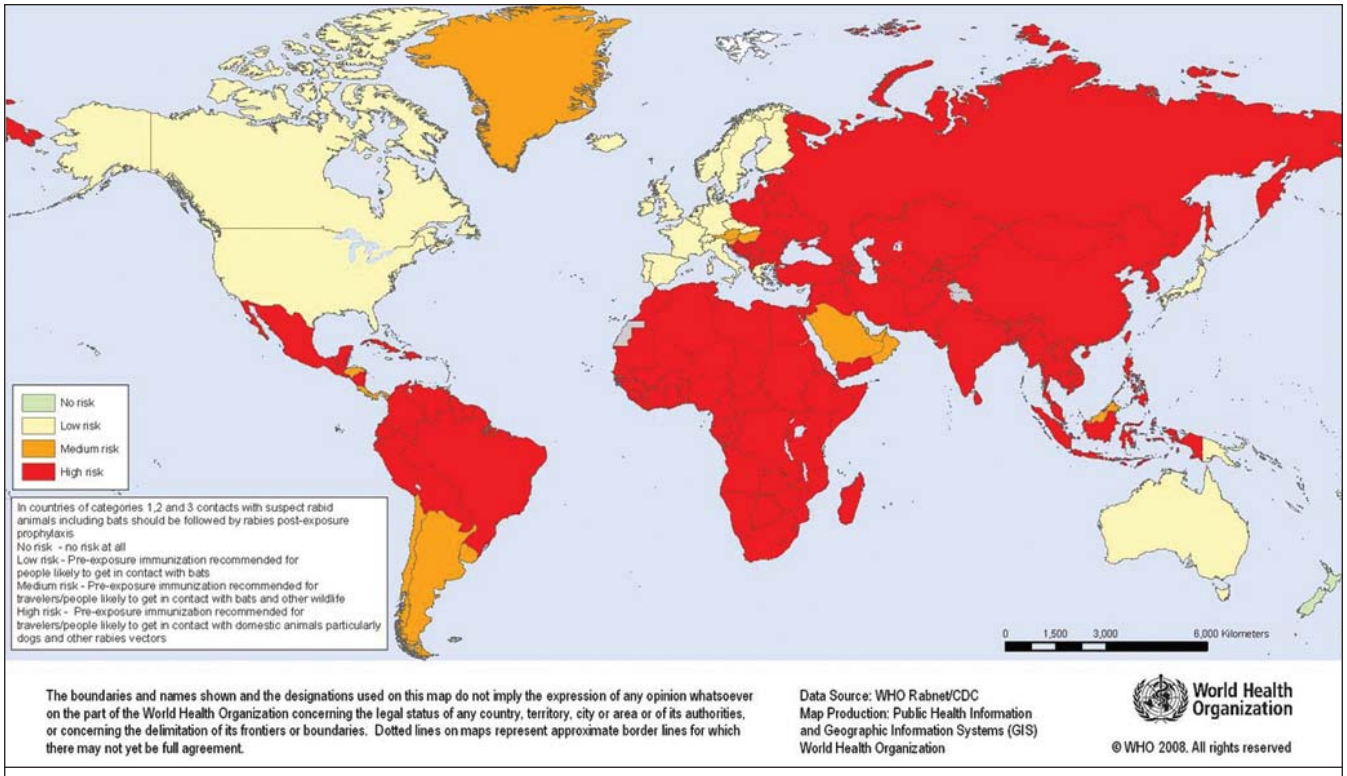
BAT RABIES IN THE UNITED STATES

Bat rabies was recognized for the first time in the United States in 1953. The son of a migrant worker from Mexico was bitten by a bat while searching for a lost ball on a ranch near Tampa, Florida. The bat was knocked to the ground by the boy's mother, and killed by the father. Fortunately, the owner of the ranch knew about rabies in vampire bats in Mexico and insisted that the bat be tested for rabies. It was sent to the local public health laboratory and was confirmed to be positive through the identification of Negri bodies on microscopic examination. Further testing included inoculations of mice which resulted in the 5 injected mice dying with clinical symptoms of rabies. The bat was later identified as a lactating female Florida yellow bat (*Dasypterus floridanus* Miller, 1902). The boy was administered postexposure prophylaxis and, after an initial period of illness, recovered uneventfully.[21]

The following year (1954), a study was undertaken at the US Army Veterinary Laboratory at Fort Sam Houston to investigate the prevalence of rabies in bat species throughout the southwestern United States. A total of 1,247 bats were collected from various sites in Texas, Louisiana, Arkansas, and New Mexico, including 27 vampire bats collected from 2 sites in the northern parts of Mexico for comparison. The bats were necropsied and Sellers stained impressions of brain tissue were examined to look for the presence of Negri bodies. A portion of each brain was also injected intracerebrally into Swiss albino mice to isolate the viral agent. Additionally, serologic studies were conducted using the mouse inoculation procedure. Four species of bats, 3 from Texas and one from Louisiana, were shown to be naturally infected with rabies. This study also demonstrated that a high percentage of the bat brain tissues from which rabies virus was isolated through the use of mouse inoculation had been negative for Negri bodies.[22]

BAT RABIES IN CENTRAL AND SOUTH AMERICA

There has been an increase in the number of human cases of rabies transmitted by bats throughout Latin America.[23] This has come at a time when canine rabies has begun to decline in some areas due to concerted antirabies campaigns. Of the bat cases, the majority are due to vampire bats. It has been surmised that the vampire population has increased over the past several decades due to increasing numbers of cattle operations throughout the region. In areas where the cattle have overgrazed an area and human occupations moved in, the bats simply change their food source from bovine to human, leading to outbreaks of rabies. Other factors involved include the clearing of rain forest cover, poor condition of available housing, and lack of access to health



The levels of the risk of rabies within countries and areas worldwide as of 2008. Source: http://www.who.int/rabies/resources/en/

care. In 2009, a case of rabies encephalitis in a teen aged male in Colombia was considered to be the first case of bat transmitted rabies in an urban area in the region.[24] The virus was found to be the antigenic variant whose main reservoir is the vampire bat, *Desmodus rotundus* (E. Geoffroy, 1810). The Milwaukee Protocol had been attempted, but was not successful due to a number of complicating factors.

RABIES WORLDWIDE

As shown in the Figure, rabies is present worldwide on all continents except Antarctica.[9] Terrestrial rabies, or rabies in mammals other than bats, can be found in over 150 countries. Countries that may be referred to as "rabies free" include Australia, Guam, Iceland, Japan, Norway, Sweden, and the United Kingdom. The World Organisation for Animal Health considers a country to be rabies free for international trade purposes when certain criteria are met.[25] These criteria include having a system for surveillance and notification of rabies, effective importation procedures and other measures. There can be no cases of rabies acquired within the country in animals or man for 2 years and no cases of rabies from imported carnivores outside of a quarantine station. The criteria do not include the isolation of a bat lyssavirus, which can still present as rabies disease in humans. At times a country's claim to be rabies free can be tenuous due to disease reintroduction or emergence. According to the World Health Organization, all of Africa and Asia, Central America, the northern half of South America, and the Caribbean islands are at high risk from rabies. The CDC has estimated the rate of possible exposure to rabies of travelers throughout the world to be in the range of 16 to 200 per 100,000 travelers.[26] Guidelines for rabies treatment to include both pre- and postexposure prophylaxis are specifically intended for use within the United States. These guidelines are currently the best available for deployed environments around the globe, but it must be realized that rabies is a disease which still has many potential unknowns. Rabies virus is the most important of 11 known lyssaviruses globally.[27] The other lyssaviruses cause disease which clinically resembles rabies, however, there is reason to question the effectiveness of pre- and postexposure prophylaxis based on the genetic distance each virus has from the rabies genotype.[28]

RABIES TESTING

The animal suspected of carrying rabies must be euthanized in a humane manner such that the biological integrity of the brain is not compromised. The head should be removed and transported to the laboratory in an insulated box with ice packs. Transportation should be arranged to effect the shipment of the sample to the laboratory as quickly as is feasible. A bat may be submitted as a whole body. The gold standard test for rabies in animals remains the direct fluorescent antibody test

(DFA). This test replaced the use of Sellers stain to determine the presence of Negri bodies in the mid 1960s. In the DFA test, brain tissue is adhered to glass slides in an "impression smear," and, after fixation in acetone, stained with a fluorescent conjugate. The presence of punctate bodies of apple-green fluorescence indicates a positive sample. The limitations of this test in an austere environment are the requirements for a fluorescent microscope and cold storage capability for reagents and for transport of samples.

In 2008, Veterinary Laboratory Europe, now US Army Public Health Command Region – Europe, deployed a veterinary pathologist to Iraq and Afghanistan to provide rabies surveillance capabilities and training to military personnel and key local civilian veterinarians.[29] The US military exchanged ideas and information with their civilian counterparts and shared their common interest in future coordination regarding rabies surveillance in Iraq and Afghanistan. In 2010, a veterinary officer with the 734th Agribusiness Development Team stationed in Kunar Province, Afghanistan, provided continuing education training on rabies and other topics to the private veterinary practitioners in Kunar.[30] This was done as part of an effort to establish a rabies control program for Afghanistan.

VACCINATION PROGRAMS AND SEROLOGY FOR RABIES ANTIBODY

A reccurring theme in public health seems to be the need to reeducate the public on the need for public health programs. The safety and efficacy of vaccines seem to be particularly contentious issues. In 2007, the CDC declared the United States to be free of the canine strain of rabies,[31] indicating that the rabies variant whose natural hosts include dogs and coyotes is no longer considered enzoonotic in the continental United States. This was after many decades of vaccination programs through local and state health agencies across the country. At each stop, the US Public Health Service had to convince local community health decision-makers to allow the program to go forward, often against dissenting voices in the community. A modified version of the Semple phenol killed virus vaccine was recommended by the US Army in the 1940s for use on dogs, but it required semiannual vaccination.[32] Vaccine and vaccination protocols have greatly improved since then. It is important to understand, however, that even though the canine variant may no longer be enzootic, rabies variants from bats, raccoons, and skunks still exist in the United States, and there is always the risk from imported cases of rabies.

In 1988, the canine strain of rabies was spreading in the coyote population in Starr county, Texas.[33,34] The epizootic was spreading through 11 other counties in south Texas, approaching San Antonio. A young boy died from rabies transmitted by a puppy, which had received the virus from a coyote. A new program was initiated by the Texas Department of Health (TDH) to vaccinate the coyote and later the fox populations through the use of oral bait.[35] Since 1995, the FADL Diagnostic section has evaluated coyote and fox serum by Rabies Fluorescent Focus Inhibition Test (RFFIT) as a means of monitoring the efficiency of vaccine baiting strategies in the field. The use of titer information has allowed the TDH to adjust flight plans, distribution rates, and other variables to increase effectiveness and efficiency. Each January, bait is dropped over an area of south Texas. In March or April, a cohort of animals is harvested and bled. Samples of serum are sent to the FADL for RFFIT, while other samples are retained by the TDH or sent on to the US Department of Agriculture or the CDC for related research. The program has greatly reduced the risk of transmission of rabies from coyotes and fox to domestic animals and humans.[36]

The RFFIT assay is also used by FADL to evaluate the titer of veterinarians, animal handlers, and other personnel with potential rabies exposures. This helps ensure the safety of having adequate titers of antirabies antibody, while reducing the risk of vaccine-related events by reducing the frequency of vaccination. A side benefit is the inherent cost savings. The CDC Advisory Committee on Immunization Practices recommends that personnel with animal exposure have serum antibody titers tested every 2 years.[4] Those working directly with the rabies virus should be tested every 6 months. The FADL laboratory is accredited by the American Association for Laboratory Accreditation (A2LA) to the ISO 17025 standard, and by COLA laboratory accreditation (http://www.cola.org) for human antirabies antibody testing by the RFFIT method. The FADL laboratory tested 9,620 samples by RFFIT assay from January 2000 through December 2011.

Animal travel has become a major concern for the Department of Defense. Since 1997, the FADL has offered the Fluorescent Antibody Virus Neutralization (FAVN) assay for rabies antibody testing for military working dogs as well as pets of service members going overseas. The FADL is one of only 2 laboratories in the United States that offer this test, the other being at Kansas State University. This testing has allowed the movement of animals to Hawaii, Britain, Europe, Japan, Guam, and other countries without the stress of prolonged quarantine. The laboratory is accredited by A2LA and the French Agency for Food, Environmental, and Occupational Health and Safety, the accreditation laboratory in Nancy,

France, which authorizes the results for international travel to Europe. The FADL laboratory tested 88,545 samples by the FAVN test from 1999 through 2011.

FERAL ANIMALS SHIPPED FROM IRAQ

When US forces entered Afghanistan in 2001, General Order Number 1 prohibited military members from any contact with stray dogs. Over time, some military personnel began to ignore the order and "adopt" strays as pets. In June 2008, an international animal rescue group imported a shipment of 26 animals from Iraq to Newark, New Jersey.[37] The 24 dogs and 2 cats were to be reunited with those personnel who had befriended them in theater. On arrival, one cat was found to be ill with neurologic signs. The cat was euthanized and tested negative for rabies. Three days later, an 11-month-old dog became ill. It was taken to a veterinarian and hospitalized with fever, diarrhea, wobbly gait, agitation, and crying. The dog's condition proceeded to deteriorate until it was euthanized on June 11. Testing at the New Jersey Public Health and Environmental Laboratory proved positive for rabies. Further testing at the CDC typed the virus as a variant associated with dogs in the Middle East. The dog had reportedly lived with a Soldier in Baghdad for 7 months and was not vaccinated for rabies before transport. None of the 24 dogs had the required valid rabies vaccination certificates. Five of the dogs had a previous rabies vaccination, the remaining 21 animals received their primary rabies vaccine upon embarking transport. By the time the positive rabies results were reported, the 23 dogs and one cat were spread out to California, Colorado, Connecticut, Iowa, Kentucky, Maryland, Massachusetts, Missouri, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Texas, Virginia, and Washington. State health departments in all 16 states were notified, with the recommendation that the animals receive immediate vaccine and be quarantined for 6 months. Additionally, postexposure prophylaxis was recommended for 13 individuals who were identified as having had potential exposure to infectious saliva.[37]

RABIES RISK AWARENESS

Murray and Horvath[38] outlined 6 components of deployment medical preparation the US military uses with the goal of preventing infectious diseases: preparation, education, personal protective measures, vaccines, chemoprophylaxis, and surveillance. While each component is critically important, education of rabies awareness is one in which personnel at all levels of responsibility can assist. The salient features of this approach are to stress the reasons to not pet or feed animals, especially dogs; avoid direct contact with animals and animal products; and not adopt stray animals as pets. In recognition of human nature and the individual's desire to seek the human-animal bond, all personnel must be made fully aware of the risks involved in the choices they make. It is the responsibility of military public health professionals to educate service members at all levels about the risks to health and safety in deployed environments. Although all military personnel deploying overseas receive a predeployment medical threat brief which includes rabies risk information, additional direct emphasis stressing the significance of rabies disease in wild animal populations should convince young Soldiers that their personal decisions with regard to local animals could be a matter of life or death.

The Global Alliance for Rabies Control (GARC) is a partnership of many international agencies with an interest in rabies, including the World Health Organization and the World Organization for Animal Health. The GARC has designated each September 28 as "World Rabies Day." This initiative brings together many different entities to raise awareness about the impact of human and animal rabies, prevention strategies, and efforts toward elimination of this disease from its main sources. The World Rabies Day organization (www.worldrabiesday.org) is a good source of information about educational materials in many languages for a variety of intended audiences. The rabies information page on the US Army Public Health Command website (http://phc.amedd.army.mil/topics/discond/aid/Pages/Rabies.aspx) also has helpful educational materials for rabies risk awareness, including specific information for deployed environments. The availability of such information provides the opportunity for all US military personnel, wherever they may be, to become actively engaged in the prevention of contracting this universally fatal, but entirely preventable disease.

THE CHALLENGE

At the 2012 Military Health System Conference, The Surgeon General of the Army, LTG Patricia D. Horoho, acknowledged the death of the Soldier to rabies[39]:

> Are we good enough when we lose one Soldier to a preventable illness? Last summer, an active duty Soldier died of rabies. Of rabies? This is the first active duty rabies death in over 40 years. So my question to you is… are we good enough? My challenge and my personal belief is that…we can be better! We must be better!

She continued to stress that the Military Health System must find ways to influence behaviors of patients in the "white space" between the 100 minutes a year that an average Soldier has contact with a healthcare provider.

Rabies is an entirely preventable disease if the individual is informed enough to understand the importance of (1)

avoiding contact in the first place, and (2) seeking and receiving treatment if contact occurs. The consequences otherwise are tragic.

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